II *Phycomyces Blakesleeanus*
Positive growth effect = both intermediates
No growth effect = pyrimidine alone or thiazole alone or no vitamin B_1 nor intermediate See III

III *Pythium polycladon*
Positive growth effect = pyrimidine alone
No growth effect = thiazole alone or no vitamin B_1 nor intermediate See IV

IV Tomato root or *Mucor Ramannianus*
Positive growth effect = thiazole alone
No growth effect = no vitamin B_1 nor intermediate

[1] William J. Robbins and Mary A. Bartley, *Sci.*, **85**, 246–247 (1937). William J. Robbins and Mary A. Bartley, *Proc. Nat. Acad. Sci.*, **23**, 385–388 (1937).

[2] When the terms, thiazole or pyrimidine, are used in this paper, the 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromo-methyl-6-aminopyrimidine is meant. These intermediates have been used in the synthesis of vitamin B_1.

[3] William J. Robbins and Frederick Kavanagh, *Proc. Nat. Acad. Sci.*, **23**, 499–502 (1937).

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A CYTOLOGICAL STUDY OF COLCHICINE EFFECTS IN THE INDUCTION OF POLYPLOIDY IN PLANTS

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A series of colchicine treatments of plant tissues was begun by the writer as an independent investigation in February, 1937, while employed at the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y. In a report at the annual winter meeting of the A. A. A. S. in 1936, Allen[1] mentioned that colchicine influenced mitotic activity in animal tissue. Soon thereafter Mr. E. L. Lahr,[2] a colleague in the

Department of Genetics, showed the writer some preparations of animal germinal tissue treated with colchicine previous to killing, in which mitotic figures were more abundant than in the prepared sections from untreated tissue. This observation suggested many possibilities.

It was recalled that Kostoff[3] working with amphidiploids stated that he had employed various chemical substances in treating plant tissue. His work and similar studies by others had been reviewed by the writer in a thesis[4] submitted several years previously. With these investigations as a background and an active interest in a polyploid series of plants, the Resedaceae,[5] it was natural for the writer to have an interest in any substance which might seem to offer the possibility of affecting the mitotic process. A small quantity of colchicine was obtained and a series of more or less successful preliminary experiments with plant tissues was begun.

Of course, onion root tips, radish and corn seedlings, with large nuclei and readily stainable chromatin offered distinct advantages for this type of investigation, so that most of the preliminary work up to the end of April, when the writer left Cold Spring Harbor, was done with these materials.

As the work progressed the main objectives of this initial cytological study using colchicine became centered around the following points: (1) the effect of colchicine upon individual embryonic cells rather than entire tissues; (2) the degree of polyploidy in a cytologically changed nucleus and the relation of the change to the method of treatment; (3) observable cytological changes due to the effects of colchicine upon the mitotic process; (4) the probable effectiveness of colchicine as a means of producing hereditary changes.

Since the cell reacts to the colchicine environment, independent of adjacent cells, it must be studied as an independent functional and structural unit. Therefore it is vital to consider the cytological effects upon individual embryonic cells of stem and root meristems.

Stem and root meristems of germinated *Zea mays*, *Raphanus sativa* and bulbs of *Allium cepa* were used to study the effect of colchicine upon their embryonic cells. The production of cytogenetical changes was found to be dependent upon three factors of the treatment, namely: (*a*) the concentration of colchicine solution, (*b*) the time allowed for the solution to act upon the meristem and (*c*) the physiological activity of embryonic cells at the time of treatment.

It is well known that embryonic cells are characterized by their ability to undergo mitosis and produce new cells. When meristematic cells divide mitotically the chromosomes organized from chromatin undergo equational longitudinal division into daughter chromosomes; these daughter chromosomes are completely separated from each other in metaphase, migrate to opposite poles in anaphase and subsequently reorganize as daughter

nuclei. It is also well known that the mitotic spindle formed during the process functions in the separation of daughter chromosomes and plays an important rôle in the formation of a cell plate between the daughter nuclei. The cell plate marks the place where the new cell wall is laid down to separate the two daughter cells and thus completes the mitotic process. The two new cells are genetically similar to each other and to the original parent cell. Interference or interruption of any one phase or several phases of the mitotic process could bring about the production of cells that differ from each other or from the parent cell. The colchicine treatments interrupted or inhibited certain phases of the mitotic process and without apparently affecting certain others.

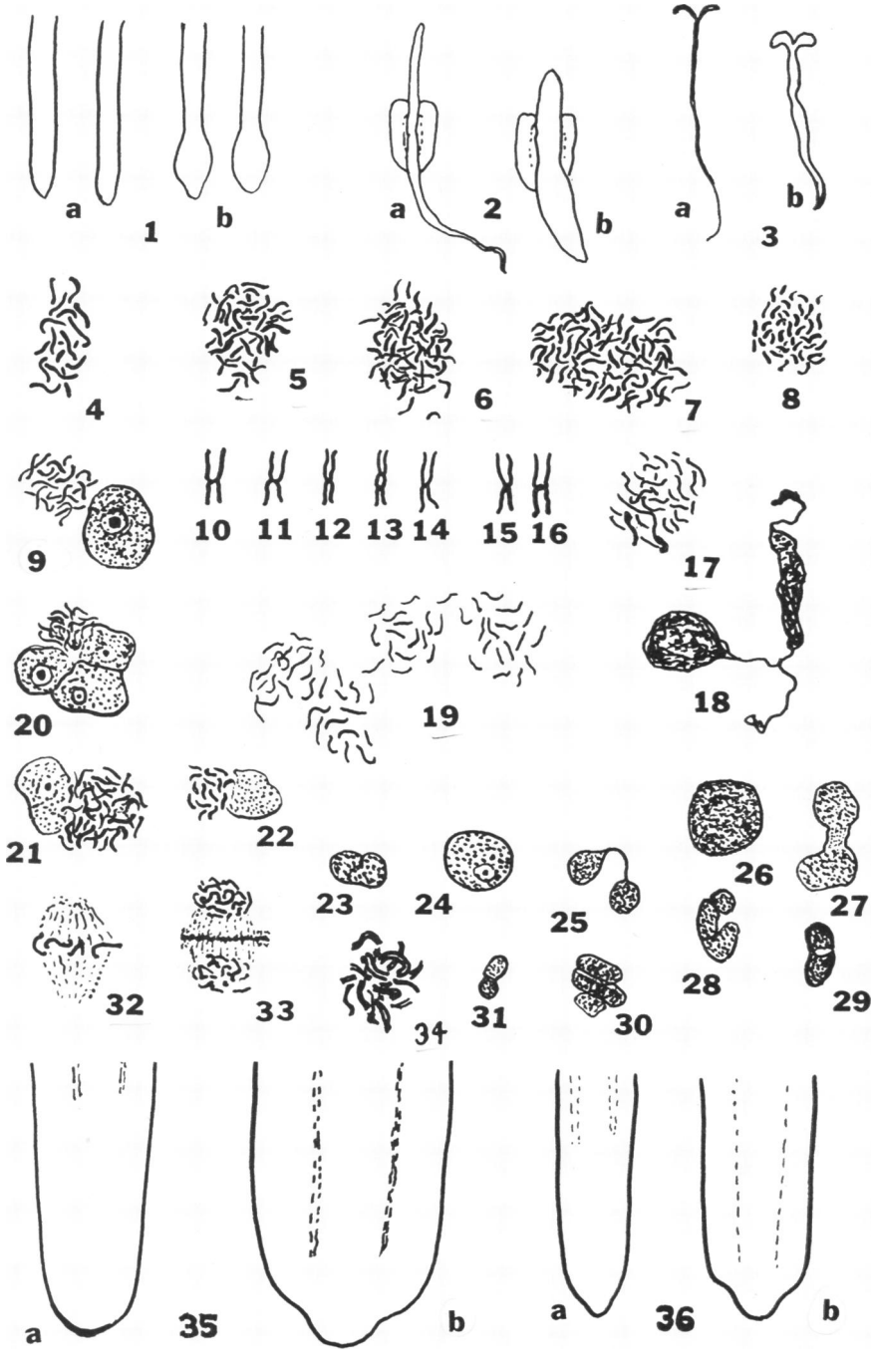
Colchicine is soluble in water and effective in very dilute solutions. The solutions used in these original studies were in concentrations of 1%, 0.1% and 0.01% and applied for 24, 48, 72, 96 and 108 hours. The seeds were germinated upon moist filter paper soaked with the desired concentration of colchicine for a given interval of time. *Allium* bulb root tips were treated by immersion. Definite toxic effects were observed from the highest concentrations. An increase in colchicine concentration or an increase in length of time for treatment beyond 72 hours was found to be detrimental and fatal to the future development of the meristematic tissue. There was a close correlation between the production of morphological abnormalities and the intensity and duration of the treatment. Increase in concentrations and increase in time for treatment caused increases in both structural abnormalities and cytological abnormalities.

Stem and root meristems were analyzed for cytological changes by two methods, namely, the aceto-carmin smear method, and the paraffin method for preparation of permanent slides. A weak Flemming solution was used for fixation and several different cytological stains were used for staining the sections. The principal plants used in this study were *Allium cepa*, *Raphanus sativa*, *Zea mays* and *Triticum vulgare*. The general morphological and tissue changes which appeared in these stem and root meristems were all similar in character. Since *Allium* root meristems offered material with large nuclei which were of advantage for cytological study this form was used most extensively for the detailed cytological studies given here.

A 0.1% and 0.01% colchicine solution both produced enlargements of *Allium* root, and stem portions, figures 1b, 2b, 3b, were consistently larger than untreated material, figures 1a, 2a, 3a. A decrease in the concentration of colchicine solution increases the length of time necessary for production of the enlargement. A fairly concentrated solution of 1% has

EXPLANATION FOR FIGURES

Figure 1.—Treated and untreated *Allium*. Figure 2.—*Zea mays*. Figure 3.—*Raphanus sativa*. Figures 8, 22, 31 and 36, *Zea mays*. Other figures *Allium*. Cytological figures Mgn. × 650.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only a toxic effect, it does not produce enlargement even though the treatment is applied for sufficient time to produce change. The first sign of enlargement was found in the region of elongation some distance behind the dividing embryonic cell region. As the enlargement increased and time of treatment was prolonged, the bulbous portion came closer to the tip of the root.

This observation of enlargement over several days showed that as the swelling continued it approached the very tip of the root, and a similar progression of vascular elements were differentiating much nearer to the tip in treated roots, figures 35b, 36b, than in untreated root tips, figures 35a, 36a.

A section through one of these roots, figures 35b, 36b, shows this same progression in the differentiation of the vascular elements as compared with the untreated root tips, figures 35a, 36a.

The treatment is specific for individual cells, producing one type of change in a particular cell while the neighboring cell may be affected differently. This cytological variation from cell to cell was a characteristic feature. Such phenomena afford a basis for sectoral mutation as root tips and stem tips differentiate from the growing point.

The relationship of individual cellular reaction to the effects of colchicine, independent of adjacent cell activity is correlated with our knowledge of the individuality of mitotic activity found restricted to the activity of the cell and not the entire tissue. The enlargement of the tissue as a member is caused by increase in cell size rather than increase in cell numbers.

The chromosomes undergo longitudinal equational division, shown by a study of isolated chromosomes in aceto-carmin smear preparations, figures 10–16, which were made from colchicine treated root meristems of *Allium*. Colchicine does not inhibit or interfere with this process of chromosome division. The separation of the chromosome halves was complete except for a proportion of the achromatic region which seems to be associated with the constrictions of chromosomes. This phase of the mitotic process (longitudinal equational division of the chromosomes) must occur to produce polyploid nuclei, and during or after a moderate colchicine treatment this process continues without interruption.

The mitotic spindle or the formation of an achromatic figure was definitely inhibited in colchicine treated material as shown by chromosomes plates in aceto-carmin preparations of *Allium* in figures 5, 6, 7, 17 and 19. A paraffin section which was specifically prepared with weak Flemming solution, figure 34, and stained, with the triple stain, failed to show spindle formation. Similar material untreated and fixed in Flemming solution, and similarly stained, showed the presence of the spindle and chromosomes in a definite equatorial plate, figure 32, and a later mitotic

stage, figure 33, showed development of the spindle and cell plate between nuclei. From this and similar observations it was concluded that colchicine prevents spindle formation.

The diploid number of chromosomes for *Allium* is 16, shown in figure 4 from an untreated control. Counts of chromosome numbers in *Allium* after 48 hours of treatment with colchicine were 32 in figure 5; 48 in figure 6; 64 in figure 7. For the treated *Zea mays* stem meristems, figure 8, 40 chromosomes were counted. Application of pressure to the cover slips of aceto-carmin preparations made it possible to count the higher numbers. The evident cytological change was one of polyploidy. Polyploid cells could be found in both stem figures 8, 22, and root meristems, figures 5, 7, following colchicine treatment. Polyploidy of cells in the meristems of stems as well as root tissue indicates that colchicine may be valuable for the induction of polyploidy with practical significance, because these cytogenetical variations produced in the stem and transmitted by growth to the reproductive organs could be used for purposes of propagation and plant breeding.

Up to April the writer's investigation had not progressed beyond the cytological observations given in the paper, but it was felt that heretofore it had not been possible to induce cytogenetic changes by the application of reagents to embryonic cells in such an effective manner as was indicated by these preliminary studies. Researches by Nemec,[6] Winkler,[7] Jorgensen,[8] Randolph[9] and Kostoff,[3] had also shown that there were possibilities of inducing cytogenetic changes in various ways.

Blakeslee,[10,11] who made reference to the writers' unpublished results, and encouraged this publication has since proved beyond doubt that in *Datura*, tetraploids may be obtained from diploids after colchicine treatment. Likewise, Nebel[12] has been carrying out a series of cytological studies on both plant and animal tissue which indicated the same possibility. Thus it appears likely that the use of colchicine will prove to be of great practical importance to genetics.

Multinucleate structures were produced in *Allium* after colchicine treatment as shown in figures 9, 20, 21, 23, 25, 27, 28, 29, 30 and in *Zea mays* stem meristematic cells, figure 31. These conditions are probably due to prolonged treatment in solutions that were not entirely toxic. Each part of the multinucleate structure or compound nucleus represents the diploid number, the chromosomes of which are capable of dividing to produce an octoploid nucleus, figure 19. In this way octoploid nuclei are derived from tetraploid nuclei. It is possible for one part of the compound nucleus to divide, figure 9, while the other part remains in interphase. The result of this process would be a 48-chromosome or hexaploid nucleus, figure 6. The fact that polyploid nuclei have formed during treatment and that with prolonged treatment these polyploid nuclei again divided to form hexaploids

or octoploids, indicates the reason why colchicine is effective in polyploid induction. It is because this reagent does not inhibit chromosome division, but does prevent spindle and cell plate formation. The multinucleated structures are abnormal cytological monstrosities but may be regarded as components of polyploid nuclei in cells, some of which may still be capable of dividing later to form tissue. It is possible that this is the answer to the question regarding the peculiar effectiveness of colchicine as an agent.

The binucleate condition was observed in stem meristems of treated *Zea* tissue, figure 31. Another cell in *Zea* tissue showed that a portion of the nucleated component is capable of forming additional chromosomes, figure 22. The presence of conditions in stem meristems similar to root meristems indicated that principles of polyploid induction found to be true for roots were also true for stems.

The multinucleate portions adhere to each other so that separation of the compound nucleus from the cell does not break up the nucleus into its component nuclear parts. In many cells the polyploid condition is present in the form of a single nucleus. In these cases the existence of multiple numbers of chromosomes could be inferred by comparison of size of nucleus.

Induction of polyploidy by colchicine occurs in two general stages: namely (1) a stage marked by increased chromosome number without nuclear abnormalities and irregularities, and (2) the induction of polyploidy with abnormal shapes of nuclei and abnormal counts. The latter class is characteristic of cells treated for a long time and with greater concentrations of colchicine. These results were found in some cases recorded with abnormal nuclei, figures 18, 25, 27 and abnormal dividing chromosomes, figure 17. The former class marked by polyploidy without abnormalities was a group of changes produced by treatment for shorter periods of time and less concentrated solutions. It is that class of induced polyploidy which is capable of division during treatment and after treatment in which we are particularly interested, from the point of view of hereditary problems. The abnormal nuclei, figure 18, were only cytological monstrosities and more characteristic for the conditions produced by root tip treatments with chloral hydrate and other chemicals, or external environmental agents. Abnormal phases showed more fusions of chromosomes, fragments lost from nuclei and other more unusual cytological occurrences.

The physiological activity of the embryonic cell at the time of treatment is important because increase of cell activity means increase in number of mitotic divisions. This increase in mitotic division renders colchicine treatment more effective. The methods used in this study with treated seedlings already germinated and growing meristems will not yield precisely the same results when applied to the relatively inactive meristematic cells of dry seeds.

This study demonstrated that colchicine is effective in the production of cytogenetically changed cells. The process affects the mitotic divisions; hence, it is a study of independent cells as structural and functional units. These units divide mitotically at a given time and do so independently of the activity of the adjacent cells. The writer was not concerned with a study of entire tissues influenced by colchicine treatment. The exact rôle played by colchicine is essentially the inhibition of the mitotic spindle which prevents separation of the daughter nuclei, and cell plate formation, with the subsequent division into two cells. The failure of the reagent to interfere with the process of chromosome formation by longitudinal equational divisions, shows a specificity of a high degree for inhibition of certain phases of cell division and the apparent promotion of other phases of the mitotic process.

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[2] Mr. E. L. Lahr gave the writer some helpful suggestions in the initiation of this investigation.

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CHEMICAL STRATIFICATION AND LAKE MORPHOLOGY

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Data collected during an extended investigation of Linsley Pond, a small eutropic lake (area 94,400 m.^{2}, max. depth 14.8 m., mean depth 6.7 m.) near North Branford, Connecticut, throw considerable light on the nature of the water-movements in the hypolimnia of thermally stratified lakes. That such movements occur is clear from the rise in concentration of substances, that can only have been derived from the bottom mud, at distances from the latter far exceeding those over which molecular diffusion could be effective. The nature of such water movements has been a matter of discussion, three main hypotheses having been advanced in recent years. Birge,[1] Thienemann[2] and more explicitly Grote,[3] have regarded